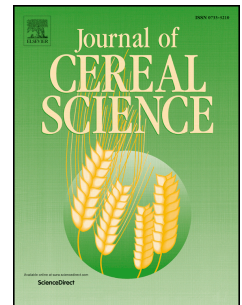


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INFLUENCE OF HIGH DAYTIME TEMPERATURE DURING THE GRAIN FILLING STAGE ON FISSURE FORMATION IN RICE

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ABSTRACT

High daytime temperatures during the grain filling stage in rice have negative impacts on milling quality traits. In this study, we used growth chambers to evaluate the influence of high daytime temperature (33°C) during grain filling, together with grain moisture content at harvest (26%, 18% and 15%), on grain fissure formation. Varietal susceptibility to fissure formation was also evaluated by exposing grains to high temperature at different grain filling stages (milky, dough, maturing). Two fissure resistant varieties: Cypress (long-grain) and Reiziq (medium-grain) and susceptible varieties: YC53-00-7 (long-grain) and Baru (medium-grain) were compared. The average HRY of Cypress declined from 62.7% at 25°C to 53.5% at 33°C, while Reiziq declined from 56.2% (25°C) to 47.4% (33°C). Both were significantly higher than the HRY of YC53-00-7 (39.2% and 24.9%) and Baru (39.3% and 31.7%) at 25°C and 33°C, respectively. When

28 grains were drier at harvest (15% cf. 26%) there was a greater reduction in HRY.
29 When the four varieties were exposed to high temperature, the highest average
30 reduction of HRY was recorded at 21 DAH. It is important to choose an optimal
31 sowing date to avoid coincidence of the final grain-filling stage with high
32 temperatures, in order to minimize milling quality losses.

33

34 **Keywords:** breakage, fissuring, milling, *Oryza sativa*.

35

36 INTRODUCTION

37

38 Rice is the staple food for more than half of the world's population (Khush, 2005).
39 Rice is processed in two steps namely de-hulling and milling and milled rice is
40 consumed as whole grains. Therefore, broken grains produced during the milling
41 process reduce the economic return to growers and millers. Broken grains are only
42 half the price of whole grain or head rice (Banaszek & Siebenmorgen, 1990).
43 Whole grain has a kernel length more than 75% of the original brown rice length.
44 Head rice yield (HRY) is defined as the weight ratio of whole grains to the initial
45 weight of rough rice, after complete milling (Aquerreta *et al.*, 2007). Grain fissure
46 formation and chalkiness have been identified as the major causes of rice grain
47 breakage during milling (Fitzgerald & Resurreccion, 2009).

48

49 Pre- and post-harvest environmental conditions, such as high temperature during
50 grain filling, grain moisture content (GMC) at harvesting and the rate of drying
51 influence grain fissure formation and chalkiness (Bautista *et al.*, 2004). The
52 number of wetting-drying cycles during final grain filling stage and storage also

53 influence fissure formation. Rice production and grain quality traits have been
54 impacted due to global warming as high temperature during grain filling shortens
55 the grain filling period and results in decreased grain weight and size (Peng *et al.*,
56 2004; Tashiro & Wardlaw, 1991a). In addition, high temperature also increases the
57 amount of chalky and fissured grains, thus reducing HRY (Wei *et al.*, 2009;
58 Yamakawa *et al.*, 2007).

59

60 Previous studies have shown that there are varietal differences to high temperature
61 during the grain filling stage for chalk and fissure formation (Cao *et al.*, 2009;
62 Lisle *et al.*, 2000; Tashiro & Wardlaw, 1991a). While chalky grain formation at
63 high temperature has been well studied (Fitzgerald *et al.*, 2009; Lisle, *et al.*, 2000),
64 little information is available on grain fissure formation at high daytime
65 temperatures during the grain filling stage.

66

67 Grain fissuring occurs as a result of moisture imbalance between the centre and the
68 surface of the grain. During rapid moisture adsorption and desorption, a higher
69 gradient of moisture is created between the centre and the surface of the grain
70 (Kamst *et al.*, 1999). The higher the moisture gradient, the greater chance that
71 fissure formation will occur. Most rice growing regions around the world have
72 high daytime temperatures (more than 33°C) during the grain filling stage
73 (Krishnan *et al.*, 2011). Identification of possible causes and tolerance mechanisms
74 are vital to develop rice varieties resistant to fissure formation, especially at high
75 temperatures. Further knowledge on critical temperature levels during different
76 grain filling stages is important to decide on optimal sowing times to minimise
77 exposure to high temperatures.

78

79 GMC at harvesting has a great impact on milling quality traits. The GMC is used
80 as an indicator for determination of the optimal time for harvesting. In many
81 situations, harvesting at 24% - 26% GMC has given higher HRY than harvesting at
82 lower GMC (Jodari & Linscombe, 1996). It is reported that when grains were dried
83 to below 18% GMC in the field but were then exposed to rain or dew before
84 harvesting, fissuring increased significantly (Lan & Kunze, 1996).

85

86 As noted, high temperature during the grain filling stage and GMC at harvesting
87 are critical factors in the fissuring of rice grains. This study had the following
88 objectives: 1) to determine the influence of high daytime temperature during the
89 grain filling stage on fissure formation in resistant and susceptible rice varieties, 2)
90 to determine the most susceptible stage of grain filling to high temperature: milky
91 (14-15 days after heading - DAH), dough (20-23 DAH) and maturing (28-30
92 DAH) and 3) to determine the optimal GMC at harvesting to minimize grain
93 fissuring.

94

95 MATERIALS AND METHODS

96

97 1. Influence of high daytime temperature and harvest GMC on fissure 98 formation.

99 Two long-grain (Cypress and YC53-00-7) and two medium-grain (Reiziq and
100 Baru) varieties were used in the study. Cypress (Blanche & Linscombe, 2009;
101 Counce *et al.*, 2005) and Reiziq (unpublished data) are known to be fissure
102 resistant, while YC53-00-7 and Baru (unpublished data) are fissure susceptible.

103

104 *Treatments:*

105 Four rice varieties (Cypress, YC53-00-7, Reiziq and Baru), two light period
106 (daytime) temperatures (25°C and 33°C), three harvesting times at different GMCs
107 (26%, 18% and 15%) and four replicates were used. The trial was conducted twice
108 (run-1 and run-2) and in each run there were two replicates.

109

110 *Plant growth phases:*

111 During the vegetative phase from sowing to flowering (heading), plants were
112 grown inside a temperature controlled glasshouse (28°/22°C, day/night). Each
113 variety per replicate consisted of nine pots (see Figure S1, showing the layout of
114 tubs in the glasshouse with pots, with the same layout used in the growth
115 chambers). Each pot (15 cm diameter) was filled with clay-loam soil and eight pre-
116 germinated seeds were placed in each pot. Sowing dates of varieties were
117 staggered to ensure synchronized flowering. After establishment, plants were
118 thinned to leave four uniform seedlings. Pots were kept in 1.5 m x 1.5 m fibreglass
119 tubs, 75 cm in height. During the growth period, recommended management
120 practices, especially control of water and application of fertilizer were adopted. A
121 nitrogen rate of 125 kg/ha (applied as urea), phosphorus (triple superphosphate) at
122 30 kg/ha and potassium (muriate of potash) at 38 kg/ha were applied. A split
123 application of nitrogen and potassium fertilizer was carried out one week before
124 panicle initiation to ensure better grain filling. The water level was maintained just
125 above the pot height. The number of days to 50% flowering in each pot was
126 recorded. There were no pest or disease incidences during the growth period and
127 uniform crop growth was observed. At heading, plants were transferred into the

control growth chambers. While the plants for run-1 were growing in the growth chambers, the plants needed for run-2 were grown as above in the glasshouse.

130

During the grain filling stage from flowering to harvesting, plants were grown inside controlled growth chambers. A split-split plot design was used with two replications (per run), where the main plot was temperature, sub-plot was variety and sub-sub plot was harvesting time. For run-2 the same controlled chambers were used, but the chamber that in run-1 was set to 25°C was set to 33°C and vice versa; this was done to minimize any random effects that might exist due to chamber effects. In all treatment units, varieties were allocated randomly.

138

Controlled growth chamber parameters:

Controlled growth chambers were used for the grain filling phase of crop growth. Both chambers were run at 60% relative humidity and light with photosynthetically active radiation (PAR) of $700 \pm 10 \mu\text{mol m}^{-2}\text{s}^{-1}$ for 10 hour period, with a period of 3 hrs for ramping-up from zero to $700 \mu\text{mol m}^{-2}\text{s}^{-1}$ and 2 hrs for ramping-down from $700 \mu\text{mol m}^{-2}\text{s}^{-1}$ to zero. The daytime temperatures of the two chambers were $25 \pm 1^\circ\text{C}$ and $33 \pm 1^\circ\text{C}$. In both chambers the dark period temperature was 8°C lower than the light period temperature.

147

148

Time of harvesting:

Harvesting was done at three time intervals after maturity, based on GMC at 1) 26% (physiological maturity), 2) 18% (on average 5-6 days later than physiological maturity) and 3) 15% (on average 10-14 days later than

153 physiological maturity). GMC was determined by taking the average of three
154 values from the top, middle and bottom of the panicles using a grain moisture
155 tester (Riceter J-series, Kett, Japan). When each experimental unit was harvested,
156 seeds from the 12 plants were bulked and 20 g of filled grains were used for
157 milling. After harvesting, the GMC was gradually reduced to 14%, in a humidity-
158 controlled (15%) and temperature-controlled (20°C) room, over 45 days.

159

160 *Milling:*

161 Milling was performed in two steps, de-hulling and polishing. Each sample (bulked
162 from 12 plants in an experimental unit) of 20 g paddy rice was de-hulled (Rice
163 Machine THU, Satake Engineering Co., Tokyo, Japan). The total amount of brown
164 rice was then milled using a laboratory-type brush mill (Rice Machine THU,
165 Satake Engineering Co., Tokyo, Japan). After milling, whole grains and broken
166 grains present in the white rice sample were separated and weights were recorded.
167 Fissured grains from the whole grain sample were identified using a Grainscope
168 (TX-200, Kett Electric Laboratory, Japan) and then separated and their weight
169 recorded.

170

171 Chalky grains present in the sample of whole grains (fissured and non-fissured)
172 were separated and weights were recorded. A visual rating was used to identify
173 chalkiness (Cruz & Khush, 2000). If a single grain had chalkiness of more than
174 10% of the total area, then it was considered as a chalky grain.

175

176

177

178 *Data analysis:*

179 Random effects of the growth chambers were estimated by analysing the means of
180 run-1 and run-2, separately. Treatment effects with non-significant chamber effects
181 were analysed by combining run-1 and run-2 (total of four replicates) using the
182 general linear model procedure from the SAS statistical software package (v6.12,
183 SAS Institute, Inc. Cary, NC, USA, 1996). Means of HRY, percentage of fissured
184 and chalky grains at the two temperatures were compared with the Tukey
185 significance test at a 0.05 probability level.

186

187 **2. Determination of the grain filling stage sensitive to high temperature.**

188

189 In addition to the above experiment (Figure S1) an additional 32 pots (as grown
190 per run) were used to determine the most sensitive stage of grain filling to high
191 temperature.

192

193 A total of eight pots per variety were initially kept in a 25°C controlled chamber at
194 heading. A single pot represented an experimental unit with two replicates (per
195 run). Two pots were transferred at a time to a 33°C temperature controlled chamber
196 at 14 (milky), 21 (dough) and 28 (maturity) DAH. The remaining two pots were
197 kept in the 25°C chamber as controls.

198

199 The experiment was repeated (run-2), under identical conditions. As in the
200 previous experiment, temperature inside the chambers was interchanged to
201 minimize random effects of the chambers. All these plants were harvested at

202 physiological maturity, 26% GMC. The total data set from four replicates (run-1
203 and run-2) was used for analysis.

204

205 **RESULTS**

206

207 **1. Influence of high daytime temperature and harvest GMC on fissure** 208 **formation.**

209

210 Plant growth duration from heading to different harvest-times where rice GMC had
211 reached 26, 18 or 15% was recorded at the two daytime temperatures, 25°C and
212 33°C (Table 1). HRY, percentages of chalky and fissured grains were recorded
213 after complete milling.

214

215 ***Head Rice Yield (HR)***

216 At both daytime temperatures of 25°C and 33°C during grain filling, Cypress (long
217 grain, resistant) and Reiziq (medium grain, resistant) produced a significantly
218 higher HRY than YC53-00-7 (long grain, susceptible) and Baru (medium grain,
219 susceptible) (Figure 1a, b). The average HRY of the susceptible varieties was
220 20.2% less than the resistant varieties at 25°C, and 22.2% less at 33°C (Table 2).
221 The average reduction of HRY for resistant and susceptible varieties was 9.2 and
222 10.9%, respectively, when daytime temperature during grain filling was increased
223 (from 25°C to 33°C) (Table 2). Thus the inherent differences in HRY between
224 resistant and susceptible varieties were greater than the differences associated with
225 temperature treatments.

226

227 The harvest GMC had a significant effect on HRY. GMC at harvesting had a
 228 greater effect on HRY in susceptible varieties than did the high daytime
 229 temperature treatment. Harvesting at 15% GMC compared to 26%, resulted in a
 230 reduction of HRY at both temperature levels during grain filling. When grain
 231 filling occurred at 25°C, the average reduction of HRY of the susceptible varieties
 232 was 19.9%, while the reduction was 9.8% in resistant varieties when harvested at
 233 15% GMC compared to 26%. At a daytime temperature of 33°C, YC53-00-7
 234 showed the highest reduction in HRY (24.1%) when harvesting was delayed
 235 (harvested at 15% GMC), while the resistant varieties showed an average of 14.1%
 236 HRY reduction with delayed harvesting (Figure 1 a & b).

237

238 *Chalky grains*

239 The percentage of chalky grains was significantly higher at a daytime temperature
 240 of 33°C (Figure 1 c & d). The highest percentage of chalky grains was observed in
 241 Reiziq under both temperature conditions, while Cypress had the lowest percentage
 242 of chalky grains (Table 3). Further, GMC at harvesting had no effect on the
 243 percentage of chalky grains.

244

245 *Fissured grains*

246 Cypress showed the lowest percentage of fissured grains at both grain filling
 247 temperature treatments (Table 4). Under stress conditions (daytime temperature,
 248 33°C and 15% GMC), all four varieties showed the lowest percentage of fissured
 249 grains (Figure 1e & 1f), compared to the optimum temperature (25°C) and GMC
 250 (26%), indicating most of the fissured grains had already broken during milling
 251 and contributed to the lower HRY at 33°C and 15% GMC.

252

253 **2. Determination of the grain filling stage sensitive to high temperature**

254

255 When the plants were exposed to a daytime temperature of 33°C during different
 256 grain filling stages, the resistant and susceptible varieties had different responses
 257 for the measured grain quality traits (HRY, percentages of chalky and fissured
 258 grains).

259

260 ***Head Rice Yield (HRY)***

261 The HRY was significantly reduced when plants were exposed to high temperature
 262 at 21 DAH, followed by 28 DAH and 14 DAH (Figure 2a). The percentage
 263 reduction of whole grains was higher in susceptible varieties than in resistant
 264 varieties. Cypress had the highest HRY of 52.7%, while YC53-00-7 and Baru had
 265 the lowest (16.7% and 16.9%, respectively) with exposure to high temperature
 266 during the dough stage.

267

268 ***Chalky grains***

269 The percentage of chalky grains was greatest when plants were exposed to high
 270 temperature at 21 DAH (Figure 2b). Cypress had the lowest percentage of chalky
 271 grains, while Reiziq and Baru had the highest percentage chalkiness after high
 272 temperature exposure at 21 DAH. Under the high temperature treatment at 21
 273 DAH, the increase in chalky grains in Baru, YC53-00-7, Reiziq and Cypress was
 274 22.2, 14.2, 15.7 and 10.4%, respectively.

275

276

277 ***Fissured grains***

278 YC53-00-7 and Baru were sensitive to high temperature at 28 DAH. The percent
 279 increase of fissured grains (compared to the control) was 9.4 and 2.1% in YC53-
 280 00-7 and Baru, respectively. However, the resistant varieties showed a non-
 281 significant ($P = 0.1199$) slight increased percentage of fissured grains, when
 282 exposed to high temperature 28 DAH (Figure 2c).

283

284 **DISCUSSION**

285

286 **1. Influence of high daytime temperature and harvest GMC on fissure**
 287 **formation.**

288

289 When exposed to the high daytime temperature treatment (33°C) grain filling took,
 290 on average for the four varieties, six days less than at 25°C (Table 1). Previous
 291 reports indicate that high temperatures during the reproductive phase reduce the
 292 grain filling duration, resulting in low grain weight and size (Kim *et al.*, 2011;
 293 Tashiro & Wardlaw, 1991b). In addition, high temperatures have been shown to
 294 have adverse effects on cooking and eating quality traits (Krishnan *et al.* 2011).

295

296 High temperature had a significant negative effect on HRY (Figure 1a and 1b). A
 297 similar finding has been reported by Counce *et al.* (2005) for varieties LaGrue and
 298 Cypress. Further, increased nighttime temperature, caused a significant decrease in
 299 grain width and length, which influenced HRY (Cooper *et al.*, 2008). Moreover, at
 300 high nighttime temperature, some chemical properties were changed and induced
 301 breaking forces (Cooper *et al.*, 2008). In our study, it was observed that the

302 susceptible varieties were much more sensitive to high daytime temperature. At
 303 high daytime temperature, starch structure is altered (without change in grain
 304 dimensions) in susceptible varieties due to an increased rate of translocations. The
 305 percentage of fissured grains and loosely packed starch granules were significantly
 306 increased at high daytime temperature. The average HRY of Cypress (62.73% and
 307 53.53%) and Reiziq (56.04% and 47.39%) were significantly higher than that of
 308 YC53-00-7 (39.15% and 24.91%) and Baru (39.32% and 31.71%) at 25°C and
 309 33°C, respectively. At high temperature, the rate of dry matter accumulation in the
 310 grain increases. This produces loosely packed starch granules in the endosperm
 311 and these loosely packed regions appear opaque in mature grains of rice (after
 312 complete milling) and are known as chalky (Yamakawa *et al.*, 2008). The increase
 313 in percentages of chalky grain were 11.2% (Cypress), 9.8% (YC53-00-7), 15.8%
 314 (Reiziq) and 15.5% (Baru) at 33°C compared to 25°C (Table 3). On the other hand,
 315 the amount of fissured grains was low at high daytime temperature (33°C)
 316 compared to the optimum daytime temperature (25°C), indicating most of the
 317 fissured grains had already broken and contributed to the lower HRY at 33°C.

318

319 GMC is a good indicator of physiological maturity. At 25°C, grains reach 24-26%
 320 moisture content within an average of 30 DAH, which is the optimum GMC for
 321 harvesting (Jodari & Linscombe, 1996). Harvesting at low moisture contents (18 or
 322 15%) can have negative effects on grain milling quality traits. HRY was greatly
 323 reduced if harvested at 15% GMC. The reduction in HRY was greater in
 324 susceptible varieties (YC53-00-7 and Baru) than in resistant varieties (Cypress and
 325 Reiziq) at 15% GMC. The reduction of HRY in susceptible varieties was
 326 approximately half that of the resistant varieties, if harvested at 15% over 26%

327 GMC at both 25°C and 33°C. In contrast, GMC at harvesting had no significant
328 effect on the percentage of chalky grains at both temperatures during grain filling.
329 The percentage of fissured grains decreased slightly with reduction of GMC. Most
330 of these fissured grains had been broken during de-hulling and milling.

331

332 Among the two stress factors, high temperature and low harvest GMC, the
333 influence of temperature was comparatively less on HRY than harvest GMC.
334 When temperature increased from 25°C to 33°C, a 5.8 and 9.5% reduction in HRY
335 was recorded in resistant and susceptible varieties respectively, at 26% harvest
336 GMC. However, the average reduction in HRY due to the decreased GMC (from
337 26 to 15%) at harvest, was 11.9 and 18.9% respectively in resistant and susceptible
338 varieties at each temperature level (Figure 1a, b). Therefore, when high
339 temperatures are unavoidable, harvesting at 26% GMC will minimize the reduction
340 of HRY.

341

342 **2. Determination of the grain filling stage sensitive to high temperature**

343

344 High daytime temperature during different grain filling stages (milky, dough and
345 maturing) had varying effects on milling quality traits. When developing grains
346 were exposed to high daytime temperature at the dough stage (21 DAH), there was
347 a significant reduction of HRY found in all four varieties. YC53-00-7 and Baru
348 were highly sensitive to high temperature, with a significant decrease in HRY at 21
349 DAH. With high daytime temperature (33°C) exposure at 21 DAH, the average
350 reduction of HRY in susceptible varieties was 31.6%, while it was 16.6% in
351 resistant varieties, compared to the control (25°C).

352

353 Water status of the developing rice grain has a direct association with various
354 biochemical and physiological changes, such as accumulation of dry matter in the
355 endosperm (Kano *et al.*, 1990). At maturity, grains hold free water until 22 DAH
356 under the optimum temperature condition (25°C) and water mobility then
357 decreases with the hardening of the endosperm (Funaba *et al.*, 2006). Under
358 optimum temperature, the endosperm slowly changes from fluid to doughy with a
359 decrease of water content and accumulation of assimilates taking place. Any rapid
360 decrease of grain free water content at the dough stage alters dry matter
361 accumulation. Brooks *et al.* (1982) reported that rapid decrease of grain water
362 content (e. g. due to exposure to high temperature), affected starch synthesis and
363 other metabolic reactions in wheat and barley. The assimilate demand from the
364 grain was greatly reduced (Savin & Nicolas, 1996) and translocation of assimilates
365 was also reduced under rapid decrease of GMC in cereals (Sultana *et al.*, 1999).
366 The impaired accumulation of dry matter results in poor milling quality traits.
367 However, high temperature exposure at the early grain filling stage (e.g. 14 DAH)
368 until harvesting had less influence on HRY, as dry matter accumulation was
369 completed at a rapid rate prior to the decrease of grain water content below a
370 critical level (change from fluid to dough). At high temperature, grain water
371 content decreases below a critical level, one week earlier than at optimal
372 temperature (Funaba, *et al.*, 2006).

373

374 Similarly, exposure of grains to high temperature at 21 DAH was correlated with
375 the formation of chalky grains (Figure 2b). The total percentage of chalky grains in
376 Baru, YC53-00-7 and Reiziq was significantly higher (average 34.4%) at 21 DAH,

377 than in Cypress (15.7%), indicating tolerance to formation of chalk in Cypress
378 when exposed to high temperature during grain filling. The highest amount of
379 chalky grains were observed when rice plants were exposed to high temperature at
380 the early grain filling stages (Tashiro & Wardlaw, 1991a).

381

382 When plants were exposed to high temperature at maturing stage (28 DAH), the
383 formation of fissured grain was significantly increased. YC53-00-7 and Baru were
384 highly sensitive to high temperature, forming grain fissures when they were
385 exposed to high temperature at the final stage of grain filling. The average increase
386 of fissured grains in susceptible varieties was 5.75%, compared to the control.
387 However, Cypress and Reiziq showed tolerance to fissure formation during all
388 grain filling stages at high temperature (Figure 2c). The average increase of
389 fissured grains was 2.6% in resistant varieties at 28 DAH under high temperature.
390 Faster moisture desorption was observed in mature grains of susceptible varieties
391 at high temperatures, suggesting that the susceptible varieties have a more
392 permeable hull (unpublished data). At a rapid rate of moisture desorption, a greater
393 moisture gradient was created between the centre and the surface of the grain
394 (Kunze, 1983), resulting in fissure formation in susceptible varieties.

395

396 The later part of the grain filling (from 21 DAH to harvesting) was more critical
397 for formation of chalky and fissured grains at high temperature. If the plants were
398 exposed to high temperature during this period, the milling quality characteristics
399 were greatly reduced. Therefore, it is important to determine the optimum sowing
400 date in order to avoid the likelihood of high temperature conditions during final
401 stages of grain filling according to the particular variety.

402

403 **CONCLUSION**

404

405 High daytime temperature during grain filling stage (e. g. more than 33°C) and low
406 GMC (15%) at harvesting had significant negative impacts on milling quality
407 traits. GMC at harvesting had a greater effect on HRY in susceptible varieties than
408 did the high temperature treatment.

409

410 High temperature exposure at 21 DAH, resulted in significantly decreased HRY in
411 susceptible varieties. Further, plants exposed to high temperature at the final stages
412 of grain filling (from 21 DAH to harvesting) also resulted in increased percentages
413 of chalky and fissured grains. Therefore, it is important to decide the date of
414 sowing which best avoids the the occurrence of high temperature during the highly
415 sensitive stage of grain filling, to maximise milling quality traits.

416

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418

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424

425

426

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Table 1. Average plant growth (days to heading, in glasshouse) and grain filling duration (in growth chambers) of 12 plants at optimum (25°C) and high (33°C) temperatures.

Varieties	Days to heading	Grain-filling duration (days)					
		25°C			33°C		
		26%	18%	15%	26%	18%	15%
Cypress	102.3 (2.6)	31.7 (3.1)	36.4 (2.7)	43.6 (2.2)	25.2 (2.6)	31.6 (2.0)	35.7 (3.1)
YC53-00-7	84.6 (1.2)	29.0 (1.5)	32.3 (1.1)	38.1 (1.4)	24.8 (2.2)	28.0 (2.4)	32.4 (1.0)
Reiziq	103.2 (1.8)	32.5 (1.8)	37.4 (2)	42.2 (1.8)	25.7 (0.8)	30.3 (1.6)	35.8 (2.0)
Baru	105.5 (3.4)	30.5 (2.7)	34.4 (2.9)	40.8 (3.4)	26.4 (3.1)	30.6 (2.7)	34.2 (3.3)

Standard deviation of the mean is in parentheses.

Table 2. HRY (%) of the four rice varieties at optimum (25°C) and high (33°C) temperatures during grain filling (average of 26, 18 and 15% GMC).

Temperature	Varieties			
	Cypress	YC53-00-7	Reiziq	Baru
25°C	62.7 ^{aA}	39.2 ^{aC}	56.1 ^{aB}	39.3 ^{aC}
33°C	53.5 ^{bA}	24.9 ^{bD}	47.4 ^{bB}	31.7 ^{bC}

Different letters represent significance levels of HRY at $P=0.05$ using Tukey significance test; lowercase letters represent differences between temperatures for a given variety (columns); uppercase represent differences between varieties for a given temperature (rows).

Table 3. The percentage of chalky grains of the four rice varieties at optimum (25°C) and high (33°C) temperatures during grain filling.

Temperature	Varieties			
	Cypress	YC53-00-7	Reiziq	Baru
25°C	6.2 ^{bC}	10.1 ^{bBC}	21.6 ^{bA}	14.5 ^{bB}
33°C	17.4 ^{aC}	19.9 ^{aC}	37.4 ^{aA}	29.9 ^{aB}

Different letters represent significance levels of chalky grains at $P=0.05$ using the Tukey significance test; lowercase letters represent differences between temperatures for a given variety (columns); uppercase represent differences between varieties for a given temperature (rows).

Table 4. The influence of temperature during grain filling on the percentage of fissured grains of the four rice varieties.

Temperature	Varieties			
	Cypress	YC53-00-7	Reiziq	Baru
25°C	1.9 ^{aC}	5.3 ^{aB}	4.4 ^{aB}	7.3 ^{aA}
33°C	2.4 ^{aC}	2.8 ^{bBC}	4.2 ^{aAB}	5.7 ^{aA}

Different letters represent significance levels of fissured grains at $P=0.05$ using Tukey significance test; lowercase letters represent differences between temperatures for a given variety (columns); uppercase represent differences between varieties for a given temperature (rows).

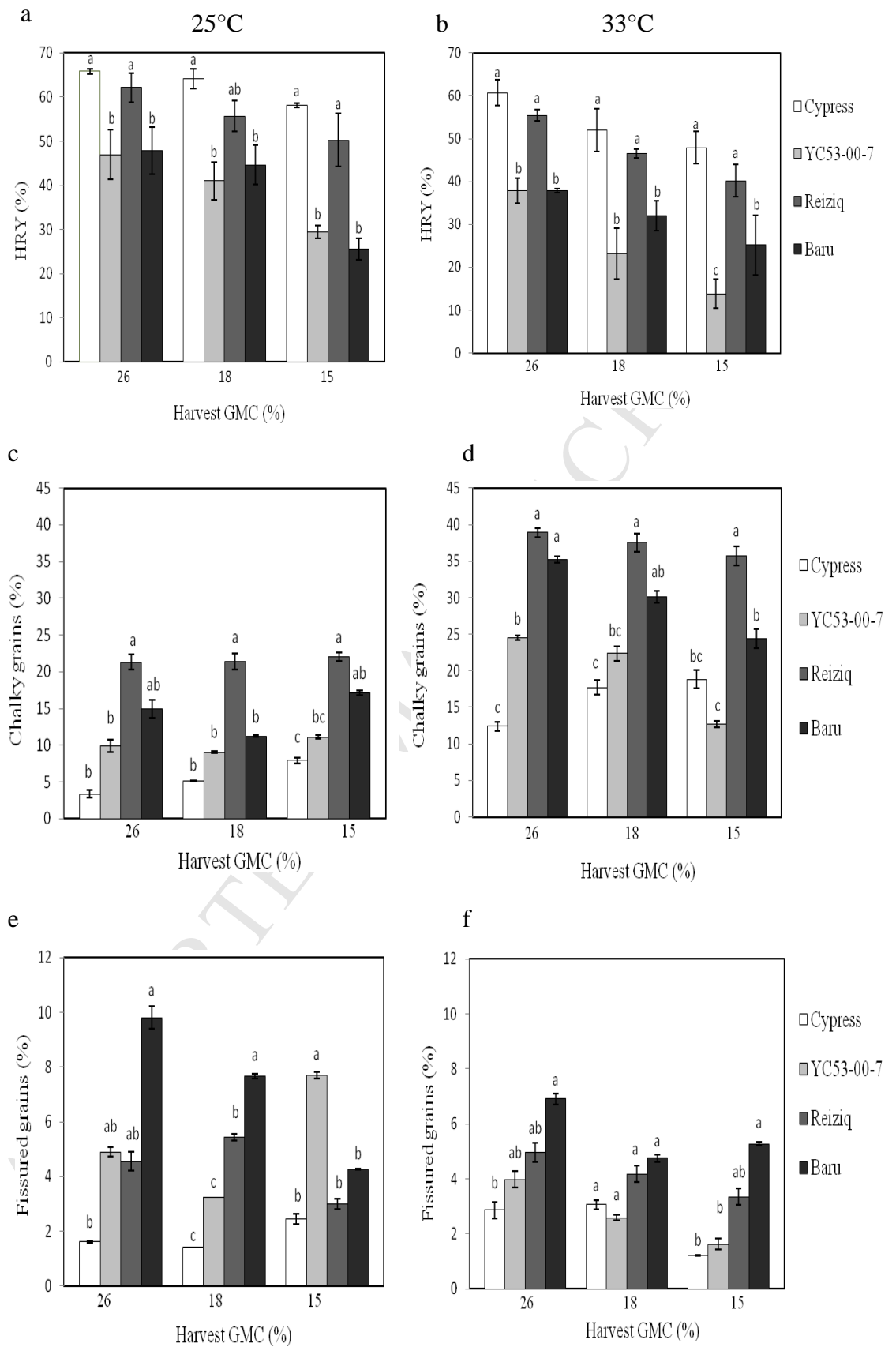


Figure 1. Milling quality traits of Cypress, YC53-00-7, Reiziq and Baru at 25°C and 33°C (left and right hand side histograms, respectively) during the grain filling stage and harvest at different GMC (26, 18 and 15%). (**a and b**) HRY, (**c and d**) percentage of chalky grains and (**e and f**) percentage of fissured grains. Means within each variety group with the same letter were not significantly different at 0.05 level probability. Error bars represent standard error.

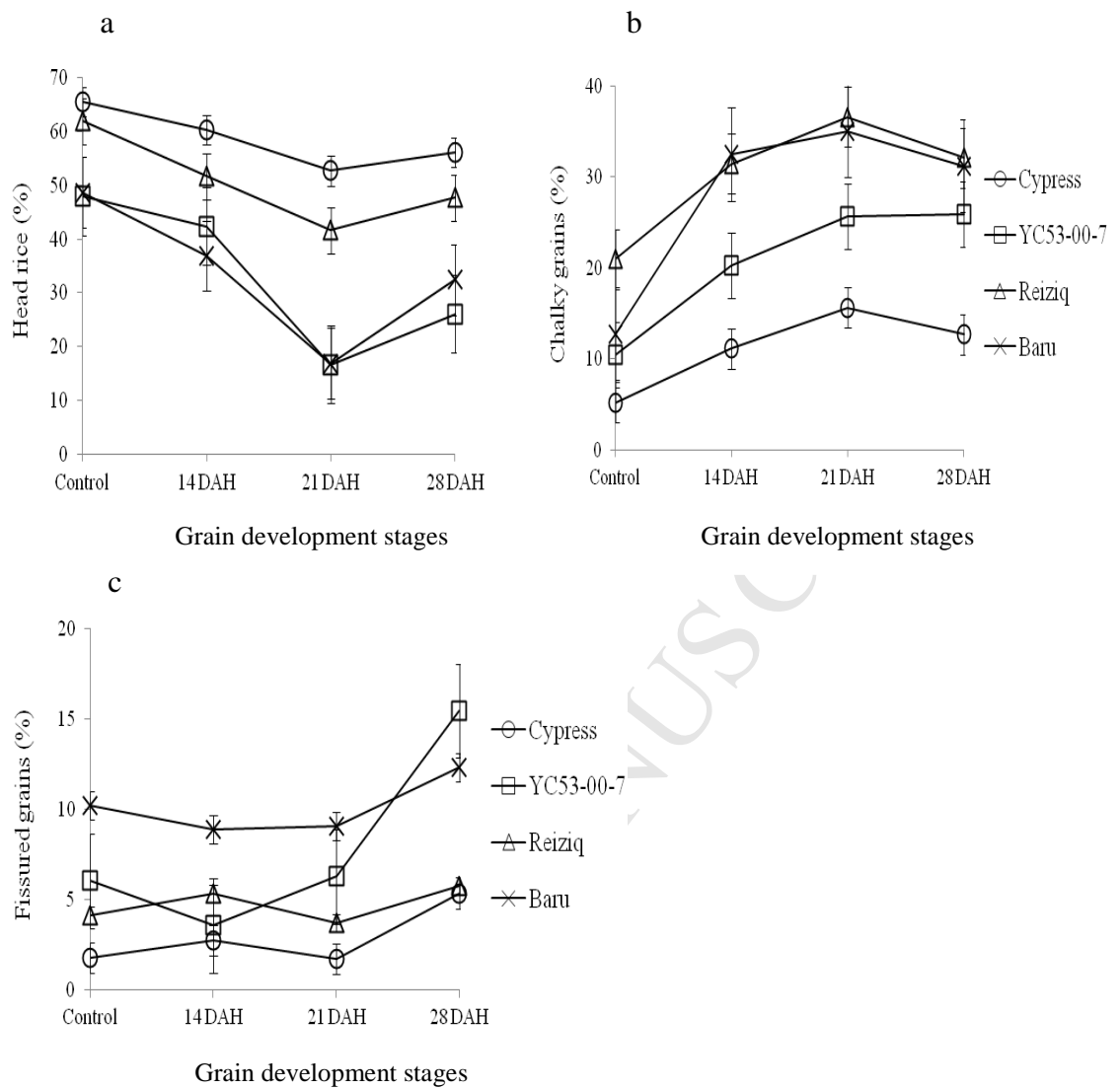


Figure 2. Milling quality traits of the four rice varieties, exposed to high temperature (33°C) at different grain development stages (14, 21 and 28 DAH). (a) percentage of head rice, (b) percentage of chalky grains and (c) percentage of fissured grains. Error bars represent the standard error.

Supplementary information

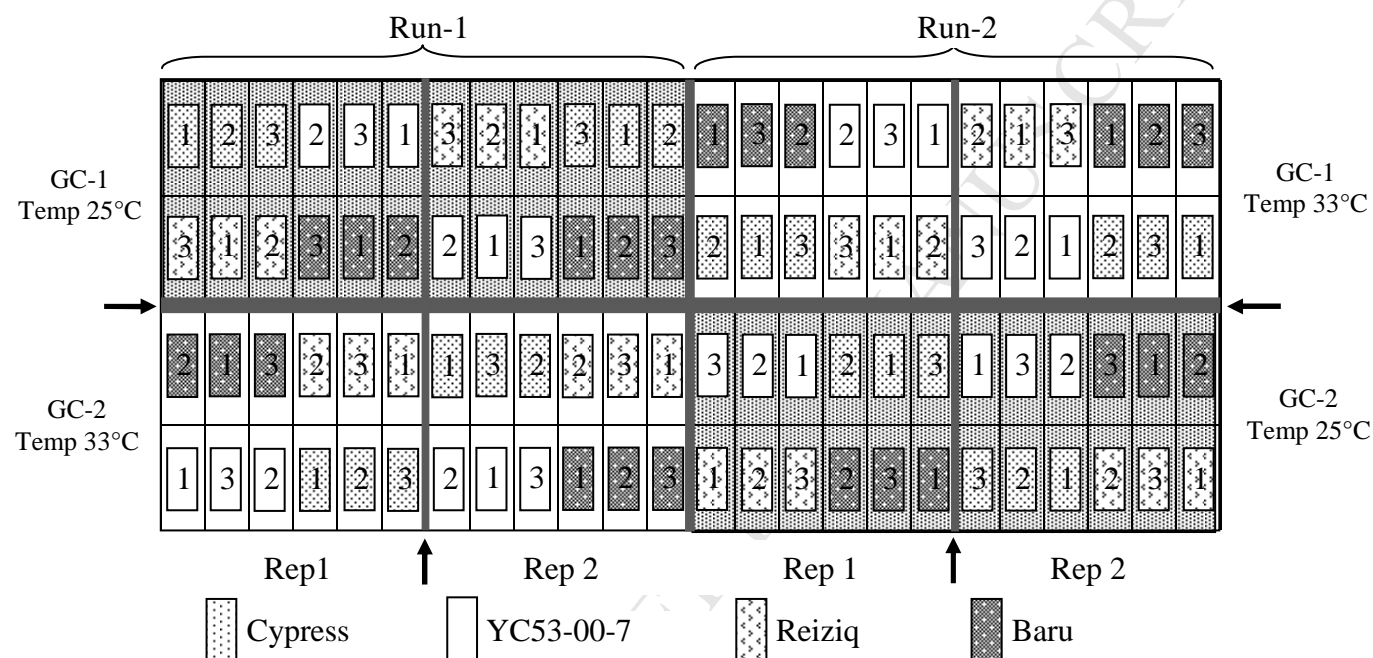


Figure S1. Schematic of randomization (split-split plot design) and time of harvesting of the four rice varieties. Temperature was used as the main plot, while varieties were used as sub-plots and harvesting time was used as sub-sub-plots. Harvesting time is represented by 1 (26% GMC), 2 (18% GMC) and 3 (15% GMC). There were three pots, each with four plants, in each experimental unit (smallest rectangles). GC=growth chamber and Temp=temperature.

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Highlights

- High temperature during grain filling and low GMC at harvesting, reduced HRY
- Effect of GMC at harvesting is greater than high temperature on HRY
- High temperature exposure at 21 DAH, resulted the lowest HRY
- Temperature sensitive grain filling stage is used deciding optimum sowing date